T-cell responses induced by ChAdOx1 nCoV-19 (AZD1222) vaccine to wild-type severe acute respiratory syndrome coronavirus 2 among people with and without HIV in South Africa

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Objective(s): This study aimed to investigate severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific T-cell responses 14 days after single-dose ChAdOx1 nCoV-19 (AZD1222) vaccination in black Africans with and without HIV in South Africa, as well as determine the effect of AZD1222 vaccination on cell-mediated immune responses in people with HIV (PWH) with prior SARS-CoV-2 infection.

Methods: A total of 70 HIV-uninfected people and 104 PWH were prospectively enrolled in the multicentre, randomized, double-blinded, placebo-controlled, phase Ib/IIa trial (COV005). Peripheral blood mononuclear cells (PBMCs) were collected from trial participants 14 days after receipt of first dose of study treatment (placebo or AZD1222 vaccine). T-cell responses against the full-length spike (FLS) glycoprotein of wild-type SARS-CoV-2 and mutated S-protein regions found in the Alpha, Beta and Delta variants were assessed using an ex-vivo ELISpot assay.

Results: Among AZD1222 recipients without preceding SARS-CoV-2 infection, T-cell responses to FLS of wild-type SARS-CoV-2 were similarly common in PWH and

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HIV-uninfected people (30/33, 90.9% vs. 16/21, 76.2%; $P = 0.138$); and magnitude of response was similar among responders (78 vs. 56 SFCs/10^6 PBMCs; $P = 0.255$). Among PWH, AZD1222 vaccinees with prior SARS-CoV-2 infection, displayed a heightened T-cell response magnitude compared with those without prior infection (186 vs. 78 SFCs/10^6 PBMCs; $P = 0.001$); and similar response rate (14/14, 100% vs. 30/33, 90.9%; $P = 0.244$).

Conclusion: Our results indicate comparable T-cell responses following AZD1222 vaccination in HIV-uninfected people and PWH on stable antiretroviral therapy. Our results additionally show that hybrid immunity acquired through SARS-CoV-2 infection and AZD1222 vaccination, induce a heightened T-cell response.

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Introduction

The nonreplicating simian adenovirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine (ChAdOx1 nCoV-19 or AZD1222), is efficacious in protecting against symptomatic coronavirus disease 2019 ('Covid-19') due to wild-type SARS-CoV-2 [1], as well as protecting against severe Covid-19 due to variants of concern. AZD1222 includes the full-length spike glycoprotein (FLS) gene of wild-type SARS-CoV-2 and induces humoral and cell-mediated immune responses after vaccination [2–5]. Cell-mediated immune responses, particularly epitope-specific T cells producing interferon-gamma (IFN-γ), can be detected using enzyme-linked immunospot (ELISpot) from day 7 and peak at day 14 following the first dose of AZD1222 [1,2,4]. No significant changes in cellular immunity have been observed between the first and second homologous AZD1222 dose, hence day 14 likely represents the peak of cellular responses [1].

The magnitude and duration of spike-specific IFN-γ producing T cells to wild-type SARS-CoV-2 elicited by AZD1222, have been shown to be similar between white European populations with HIV (PWH) and without HIV in the United Kingdom [4]. Nevertheless, there is a paucity of information on AZD1222 and other Covid-19 cell-mediated immune responses in black populations living with HIV from low-income and middle-income countries, including the effect of SARS-CoV-2 infection prior to vaccination on T-cell responses. Here we report on SARS-CoV-2-specific T-cell responses, 14 days after the first dose of study injection (placebo or AZD1222 vaccine) against wild-type SARS-CoV-2 in HIV-uninfected people and PWH in South Africa. We also report on the effect of AZD1222 vaccination on cell-mediated immune responses in those previously infected with SARS-CoV-2.

Methods

Study design and enrolment

Between 24 June 2020, and 29 July 2020, and between 17 August 2020, and 12 November 2020, 70 HIV-uninfected people and 104 PWH were respectively enrolled in a randomized, double-blinded, placebo-controlled, phase Ib/IIa trial (COV005) at two South African sites [Wits Vaccines and Infectious Diseases Analytics (VIDA) Research Unit; Wits Reproductive Health and HIV Institute (RHI) Research Centre], for intensive vaccine safety and immunogenicity monitoring [5,6]. Enrolled participants were healthy adults between 18 and 65 years with confirmed HIV statuses. Detailed inclusion and exclusion criteria of participants are described in the trial protocol [7]. PWH had additional requirements for eligibility, which included stable antiretroviral therapy (ART) for at least 3 months, and human immunodeficiency virus type 1 (HIV-1) viral loads of less than 1000 copies/ml within 2 weeks of trial randomization. Participants were fully informed about trial procedures and possible risks before providing written consent, after which they were randomly assigned (1 : 1) to the placebo group (injected with 0.9% sodium chloride per dose), and the AZD1222 vaccine group (injected with 5×5^{10} virus particles per dose). Details of the parent study, including interim results on the safety, vaccine efficacy and humoral immune responses have been published previously [5].

Determining SARS-CoV-2 molecular status and serostatus

SARS-CoV-2 infections at baseline and throughout the trial were identified with nucleic acid amplification tests (NAATs) [5]. Baseline anti-N IgG was measured using the nucleocapsid antigen of SARS-CoV-2 and run at PPD Central Labs (Zaventum, Belgium) as previously described [8]. Briefly, the Roche Elecsys Anti-SARS-CoV-2 serology test is an electroluminescence

immunoassay-based modality that allows for the qualitative detection of IgG reactive to the SARS-CoV-2 nucleoprotein in human sera.

Processing peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood samples by density-gradient centrifugation using Lymphoprep (STEMCELL Technologies, Vancouver, Canada) and cryopreserved in liquid nitrogen as previously described [4]. Cryopreserved PBMCs were thawed as previously described [9] and counted using a hemacytometer (Bright-line Improved Neubauer 0.1 mm, Sigma-Aldrich, St Louis, MO, USA) and light microscope (DM500, Leica Microsystems, Wetzlar, Germany).

Peptide antigen pools

Commercially available PepTivator SARS-CoV-2 peptide pools (Miltenyi Biotec, Bergisch Gladbach, Germany) were used as antigens for T-cell stimulation. Peptide pools Prot_S1 (Cat# 130-127-048) and Prot_S (Cat# 130-126-701) were combined to represent the FLS of wild-type SARS-CoV-2 [10,11]. Prot_S B.1.1.7 (Cat# 130-127-844), Prot_S B.1.351 (Cat# 130-127-958), and Prot_S B.1.617.2 (Cat# 130-128-763), were included to represent selectively mutated regions found in the S-protein of the Alpha, Beta, and Delta variants of concern. Three corresponding reference pools (Cat# 130-127-841, Cat# 130-127-952, and Cat# 130-128-761), containing 30–34 homologous peptides of wild-type SARS-CoV-2 (WT pools: partial S-protein regions of wild-type SARS-CoV-2) were included as controls for the variant pools.

Ex-vivo IFN-γ enzyme-linked immunospot assay

Cryopreserved PBMCs were tested in a blinded fashion using an ex-vivo IFN-γ enzyme-linked immunospot (ELISpot) assay with Human IFN-γ ELISpot^{BASIC} kits (Mabtech AB, Nacka Strand, Sweden). A total of 2–2.5 × 10^5 PBMCs were stimulated in duplicate with 1 μg/ml peptide pools for 18–20 h. Peptide pools were titrated to identify the lowest concentration that can elicit an IFN-γ response. Unstimulated negative controls and 10 μg/ml Phytohemagglutinin-L (Sigma-Aldrich) positive controls were included. ELISpot plates were read using an ELISpot plate reader (Motorized Zeiss Axio Imager M1, Carl Zeiss, Oberkochen, Germany) and KS ELISpot software (v.4.11). T-cell responses were quantified by IFN-γ-producing T cells expressed as spot-forming cells per million (SFCs/10^6) PBMCs by subtracting mean background responses in unstimulated control wells from mean responses in antigen-stimulated wells. Positive responders were defined as having more than 10 SFCs/10^6 PBMCs.

Statistical analysis

Summarized demographic and clinical characteristics are reported as medians with interquartile (IQR) ranges for quantitative variables (age and $CD4^+$ counts), and as counts with percentages (proportions) for categorical variables. T-cell responses for positive responders are summarized as geometric mean SFCs/10^6 PBMCs with 95% confidence intervals (95% CI). The chi-square test and Student's *t* test (unpaired) were used for comparing proportions and geometric means, respectively. Statistical analysis and graphical representation were performed using R (v.4.02; R Foundation, Vienna, Austria) and Prism (v.9.3.1; GraphPad Software Inc., San Diego, CA, USA), respectively. *P* values less than 0.05 were considered statistically significant.

Study approvals

The study forms part of the COV005 trial, which is registered with ClinicalTrials.gov (NTC04444674), and the Pan African Trials Registry (PACTR202006922165132). The trial's protocol (v.6.0) [7] was approved by the South African Health Products Regulatory Authority, and the human ethics committees of the University of Oxford, University of the Witwatersrand, Stellenbosch University, and University of Cape Town.

Results

We enrolled 70 HIV-uninfected people and 104 PWH in the study. T-cell responses were evaluated at day 14 following the first dose of study injection (see Figure, Supplemental Digital Content 1, http://links.lww.com/QAD/C660, which illustrates the study profile). Sixteen participants (HIV-uninfected: $n = 9$; PWH: $n = 7$) were excluded from the analysis: study withdrawal ($n = 1$), PBMCs not collected ($n = 8$), nonviable PBMCs at the time of analysis ($n = 5$), and no baseline (day 0) SARS-CoV-2 serology ($n = 2$). Ten participants (eight HIV-uninfected and two PWH) with a positive SARS-CoV-2 NAAT before or at baseline, as well as nine participants (eight HIV-uninfected and one PWH) with a positive SARS-CoV-2 NAAT between days 0 and 14, were also excluded.

The remaining 139 participants constituted of 99.3% black Africans (138/139), 69 of whom received AZD1222 (HIV-uninfected: $n = 22$; PWH: $n = 47$) and 70 placebo (HIV-uninfected: $n = 23$; PWH: $n = 47$; Table 1). Underlying comorbidities included 21.6% (30/139) with obesity, 8.6% (12/139) with hypertension and 10.1% (14/139) with chronic respiratory conditions, which were similar between HIV-uninfected people and PWH as previously described [5]. Seventy-two percent (68/94) of PWH were women and the overall median age was 40 years (IQR: 33–46); whilst in the HIV-uninfected group, 37.8% (17/45) were female with a median age of 34 years (IQR: 26–42). The median $CD4^+$ T-cell count for PWH was 680 cells/μl (IQR: 503–911) with median HIV-1 viral loads of 10 copies/ml (IQR: 10–50).

Among HIV-uninfected people who were anti-N IgG seronegative at baseline, 76.2% (16/21) and 54.5% (12/22;

Table 1. Baseline demographics for SARS-CoV-2 nucleic acid amplification test-negative participants, stratified by baseline SARS-CoV-2 serostatus and HIV status[a].

Variable	Overall	Anti-N IgG seronegative			Anti-N IgG seropositive		
HIV-uninfected people							
Number tested	**Total (*N* = 45)**	**Total (*n* = 43)**	**Placebo (*n* = 22)**	**Vaccine (*n* = 21)**	**Total (*n* = 2)**	**Placebo (*n* = 1)**	**Vaccine (*n* = 1)**
Median age, years (IQR)	34 (26–42)	34 (26–42)	33 (26–44)	34 (24–40)	34 (33–36)	31 (31–31)	37 (37–37)
Sex							
Female	17 (37.8)	16 (37.2)	8 (36.4)	8 (38.1)	1 (50)	0 (0)	1 (100)
Male	28 (62.2)	27 (62.8)	14 (63.6)	13 (61.9)	1 (50)	1 (100)	0 (0)
Race							
Black	45 (100)	43 (100)	22 (100)	21 (100)	2 (100)	1 (100)	1 (100)
BMI							
Underweight (0 to <18.5)	5 (11.1)	5 (11.6)	4 (18.2)	1 (4.8)	0 (0)	0 (0)	0 (0)
Normal (18.5 to <25)	19 (42.2)	19 (44.2)	10 (45.5)	9 (42.9)	0 (0)	0 (0)	0 (0)
Overweight (25 to <30)	11 (24.4)	9 (20.9)	2 (9.1)	7 (33.3)	2 (100)	1 (100)	1 (100)
Obese (≥30)	10 (22.2)	10 (23.3)	6 (27.3)	4 (19.0)	0 (0)	0 (0)	0 (0)
Hypertension	1 (2.2)	1 (2.3)	0 (0)	1 (4.8)	0 (0)	0 (0)	0 (0)
Current alcohol user	20 (44.4)	19 (44.2)	11 (50)	8 (38.1)	1 (50)	0 (0)	1 (100)
Current smoker	23 (51.1)	22 (51.2)	11 (50)	11 (52.4)	1 (50)	0 (0)	1 (100)
Healthcare worker	1 (2.2)	1 (2.3)	1 (4.5)	0 (0)	0 (0)	0 (0)	0 (0)
Median $CD4^+$ count, cells/μl (IQR)	NA	NA	NA	NA	NA	NA	NA
People with HIV							
Number tested	**Total (*N* = 94)**	**Total (*n* = 63)**	**Placebo (*n* = 30)**	**Vaccine (*n* = 33)**	**Total (*n* = 31)**	**Placebo (*n* = 17)**	**Vaccine (*n* = 14)**
Median age, years (IQR)	40 (33–46)	37 (33–45)	40 (36–44)	36 (31–47)	43 (40–47)	44 (40–47)	44 (33–48)
Sex							
Female	68 (72.3)	45 (71.4)	23 (76.7)	22 (66.7)	23 (74.2)	14 (82.4)	9 (64.3)
Male	26 (27.7)	18 (28.6)	7 (23.3)	11 (33.3)	8 (25.8)	3 (17.6)	5 (35.7)
Race							
Black	93 (98.9)	62 (98.4)	30 (100)	32 (97)	31 (100)	17 (100)	14 (100)
White	1 (1.1)	1 (1.6)	0 (0)	1 (3)	0 (0)	0 (0)	0 (0)
BMI							
Underweight (0 to <18.5)	5 (5.3)	4 (6.3)	0 (0)	4 (12.1)	1 (3.2)	1 (5.9)	0 (0)
Normal (18.5 to <25)	40 (42.6)	24 (38.1)	11 (36.7)	13 (39.4)	16 (51.6)	5 (29.4)	11 (78.6)
Overweight (25 to <30)	29 (30.8)	24 (38.1)	11 (36.7)	13 (39.4)	5 (16.1)	4 (23.5)	1 (7.1)
Obese (≥30)	20 (21.3)	11 (17.5)	8 (26.7)	3 (9.1)	9 (29.0)	7 (41.2)	2 (14.3)
Hypertension	11 (11.7)	6 (9.5)	2 (6.7)	4 (12.1)	5 (16.1)	5 (29.4)	0 (0)
Respiratory illness	14 (14.9)	8 (12.7)	6 (20)	2 (6.2)	6 (19.4)	3 (17.6)	3 (21.4)
Current alcohol drinker	43 (45.7)	25 (40)	9 (30)	16 (48.5)	18 (58.1)	9 (52.9)	9 (64.3)
Current smoker	31 (33)	21 (33.3)	9 (30)	12 (36.4)	10 (32.3)	5 (29.4)	5 (35.7)
Healthcare worker	2 (2.1)	2 (3.2)	1 (3.3)	1 (3)	0 (0)	0 (0)	0 (0)
Median $CD4^+$ count, cells/μl (IQR)	680 (503–911)	667 (504–907)	585 (489–852)	746 (570–940)	695 (505–916)	739 (532–920)	642 (499–874)
Median $CD4^+$ percentage (IQR)	35.6 (29.7–39.4)	36.3 (29.9–41.2)	34.8 (28.1–39.3)	36.9 (31.7–41.3)	34.8 (30.1–37.8)	34.8 (28.3–38.6)	34.8 (32.3–37.2)
HIV-1 viral load <50 copies/ml	26 (27.7)	19 (30.2)	12 (40)	7 (21.2)	7 (22.6)	6 (35.3)	1 (7.1)
ARTs							
Boosted PI + 1 NRTI	3 (3.2)	2 (3.2)	2 (76.7)	0 (0)	1 (3.2)	1 (5.9)	0 (0)
Boosted PI + 2 NRTIs	3 (3.2)	1 (1.6)	1 (3.3)	0 (0)	2 (6.5)	1 (5.9)	1 (7.1)
INSTI + 2 NRTIs	10 (10.6)	7 (11.1)	4 (13.3)	3 (9.1)	3 (9.7)	1 (5.9)	2 (14.3)
NNRTI +2 NRTIs	51 (54.3)	39 (61.9)	17 (56.7)	22 (66.7)	12 (38.7)	8 (47.1)	4 (28.6)
ART duration							
<1 year	8 (8.5)	5 (7.9)	3 (10)	2 (6.1)	3 (9.7)	1 (5.9)	2 (14.3)
1 to <5 years	24 (25.5)	20 (31.7)	9 (30)	11 (33.3)	4 (12.9)	1 (5.9)	3 (21.4)
≥5 years	35 (37.2)	24 (38.1)	12 (40)	12 (36.4)	11 (35.5)	9 (52.9)	2 (14.3)

[a]Data are *n* (%) unless otherwise stated. Data exclude participants with a positive SARS-CoV-2 NAAT before or on the day of receiving study injection (placebo or AZD1222 vaccine), as well as participants that acquired SARS-CoV-2 infection within 14 days following study injection. Denotations: anti-N, antinucleocapsid protein of SARS-CoV-2; ART, antiretroviral treatment; HIV-1, human immunodeficiency virus type 1; IgG, immunoglobulin G; INSTI, integrase strand transfer inhibitor; IQR, interquartile rage; NA, not applicable; NAAT, nucleic acid amplification test; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside or nucleotide reverse transcriptase inhibitors; PI, protease inhibitor; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

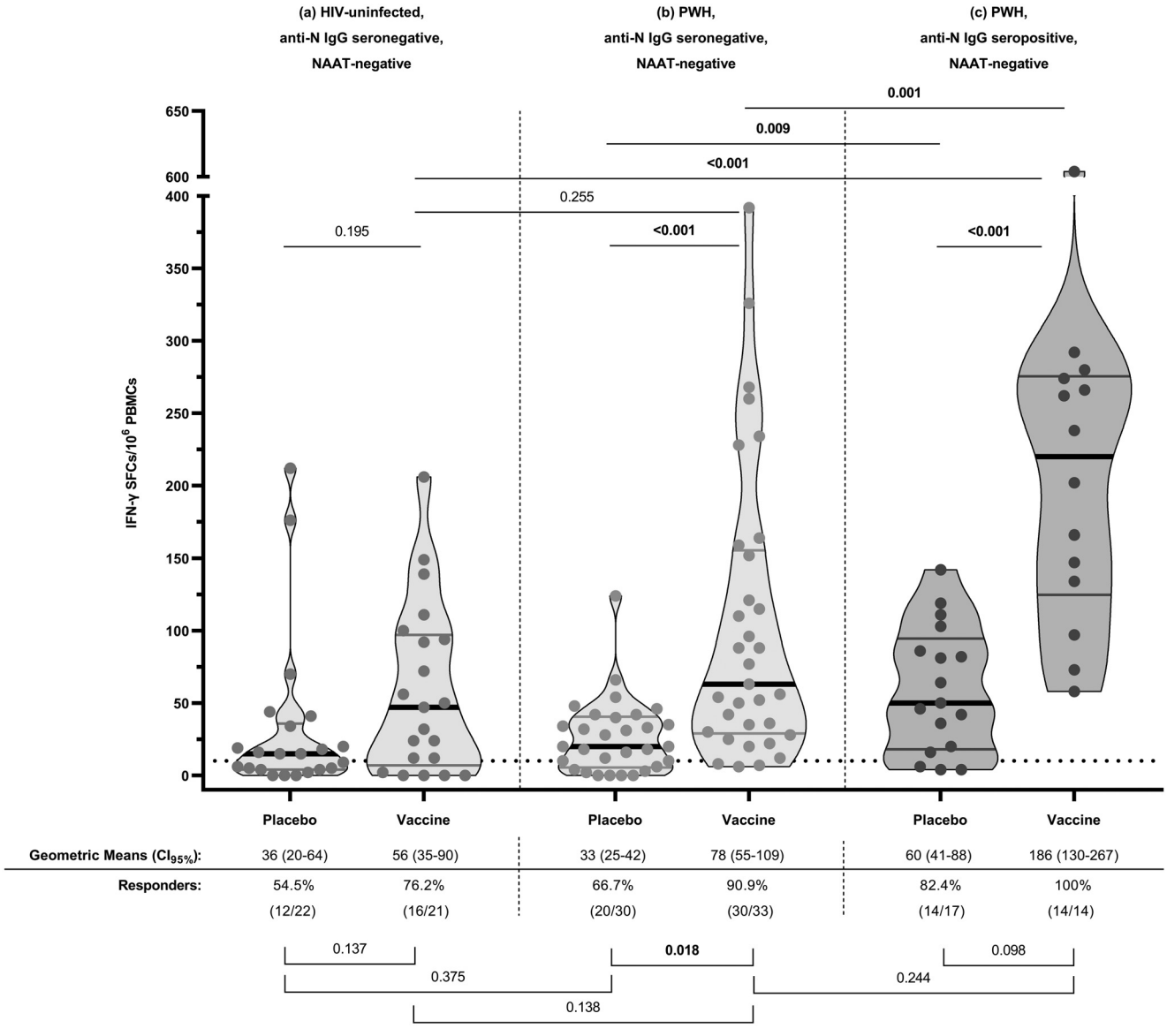

Fig. 1. IFN-γ T-cell responses against full-length spike glycoprotein of wild-type severe acute respiratory syndrome coronavirus 2 in placebo and AZD1222 recipients living with and without HIV. (a) T-cell responses in HIV-uninfected people without evidence of prior SARS-CoV-2 infection (placebo: $n = 22$; AZD1222: $n = 21$) at baseline. (b) T-cell responses in PWH without evidence of prior SARS-CoV-2 infection (placebo: $n = 30$; AZD1222: $n = 33$) at baseline. (c) T-cell response in PWH with evidence of prior SARS-CoV-2 infection (placebo: $n = 17$; AZD1222: $n = 14$). Individual points represent the total IFN-γ spot-forming-cells per million (SFCs/10^6) PBMCs of each participant. Bold black lines and coloured lines represent the median and interquartile ranges, respectively. The dotted line represents the lower limit of positive responders at 10 SFCs/10^6 PBMCs. Geometric mean SFCs/10^6 PBMCs (95% confidence intervals) were exclusively determined for positive responders using normalized data. The chi-square test and Student's *t* test (unpaired) were used for comparisons of proportions and geometric means, respectively (significant *P* values are indicated in bold). Denotations: anti-N, antinucleocapsid protein of SARS-CoV-2; FLS, full-length spike glycoprotein; IFN-γ=interferon-gamma; IgG, immunoglobulin G; NAAT, nucleic acid amplification test; PBMC, peripheral blood mononuclear cells; PWH, people with HIV; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

recipients (66.7%, 20/30; $P = 0.018$; Fig. 1b). Also, the magnitude of IFN-γ producing T cells of positive responders was higher in AZD1222 (78 SFCs/10^6 PBMCs; 95% CI 55–109) than placebo recipients (33 SFCs/10^6 PBMCs; 95% CI 25–42; $P < 0.001$). Placebo group responders were similar in HIV-uninfected people and PWH (54.5 vs. 66.7%; $P = 0.375$) who were anti-N seronegative. Among AZD1222 recipients without preceding SARS-CoV-2 infection, T-cell responses were more common in PWH compared with HIV-uninfected people (90.9 vs. 76.2%; $P = 0.138$); and magnitude of response was similar among the responders (78 vs. 56 SFCs/10^6 PBMCs; $P = 0.255$).

In PWH who had SARS-CoV-2 infection prior to study injection, all AZD1222 recipients (100%, 14/14) and 82.4% (14/17; $P = 0.098$) of the placebo group demonstrated T-cell responses to wild-type FLS (Fig. 1c). The T-cell response magnitude in AZD1222 recipients (186 SFCs/10^6 PBMCs; 95% CI 130–267), however, was higher than the placebo group (60 SFCs/10^6 PBMCs; 95% CI 41–88; $P < 0.001$) among responders. Also, PWH vaccinated with AZD1222 following prior SARS-CoV-2 infection, displayed an increased T-cell response magnitude compared with vaccinated PWH without prior SARS-CoV-2 infection (186 vs. 78 SFCs/10^6 PBMCs; $P = 0.001$); and similar response rate (100 vs. 90.9%; $P = 0.244$).

We also investigated cellular immune responses to selectively mutated S-protein regions found in the Alpha, Beta, and Delta variants. No significant differences ($P > 0.05$) were found between wild-type and variant pools, regardless of HIV-status, SARS-CoV-2 anti-N IgG serostatus, or receipt of placebo and AZD1222 (see Figures, Supplemental Digital Content 3–5, http://links.lww.com/QAD/C662, http://links.lww.com/QAD/C663, http://links.lww.com/QAD/C664, which illustrates IFN-γ T-cell responses against the Alpha, Beta, and Delta variants).

Discussion

We show that T-cell responses to FLS of wild-type SARS-CoV-2 14 days after single-dose AZD1222 vaccination was similar in black Africans living with and without HIV who were SARS-CoV-2 anti-N IgG seronegative when vaccinated. Our findings of similar T-cell responses in PWH and HIV-uninfected AZD1222 recipients among those who were anti-N seronegative at enrolment, corroborate the observations of a study in predominantly white Europeans which also investigated AZD1222-induced IFN-γ- producing T cells using the ELISpot method [4]. Furthermore, comparisons between PWH and HIV-uninfected people who received the Pfizer-BioNTech BNT162b2 mRNA vaccine and the Sinopharm BBIBP-CorV-inactivated vaccine have similarly shown comparable vaccine-induced T-cell responses [12,13].

We also show that T-cell responses following AZD1222 vaccination was heightened in PWH who had been infected by SARS-CoV-2 prior to vaccination compared with those who were anti-N seronegative at enrolment. In PWH, prior SARS-CoV-2 infection in the placebo group was associated with similar T-cell responses and magnitude of response compared with the AZD1222 recipients who were SARS-CoV-2 anti-N IgG seronegative. Also, single-dose AZD1222 vaccination following SARS-CoV-2 infection in PWH elicited a greater T-cell response magnitude compared with vaccinated PWH without prior SARS-CoV-2 exposure, suggesting an additive benefit of vaccination following primary infection. The observation of preceding SARS-CoV-2 infection inducing higher T-cell responses in AZD1222 vaccinees among PWH, is similar to that reported in HIV-uninfected people following administration of a single-dose messenger RNA vaccine (BNT162b2) in convalescent individuals [14–16].

We observed a modest T-cell response in placebo recipients who were anti-N seronegative at enrolment in PWH (66.7%) and HIV-uninfected people (54.5%); which is likely attributable to cross-reactive T cells because of exposure to endemic human coronaviruses [17]. Cross-reactive T cells have also been found in SARS-CoV-2 anti-N IgG seronegative Europeans constituting the control group who had received the meningococcal conjugate vaccine (MenACWY) in a phase 1/2 single-blind, randomized controlled AZD1222 trial [1].

We could not differentiate cellular immune responses between placebo and AZD1222 recipients using the ELISpot method; which can be considered a limitation. Our inability to differentiate T-cell responses between the placebo and AZD1222 groups, may be ascribed to the Alpha, Beta, and Delta variant pools. These pools only contained S-protein immunodominant T-cell epitopes that are affected by variant-specific mutations [Alpha variant (EPI_ISL_756151): deletion 69, deletion 70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A and D1118H; Beta variant (EPI_ISL_861709): D80A, D215G, 242 deletion, 243 deletion, 244 deletion, K417N, E484K, N501Y, D614G and A701 V; Delta variant (EPI_ISL_1718882): T19R, G142D, E156G, deletion 157, deletion 158, L452R, T478K, D614G, P681R and D950N; GenBank Reference: MN908947.3] in contrast to the entire FLS pool that can contribute more epitopes to elicit greater T-cell responses. Poly-epitopic T-cell responses induced by Covid-19 vaccines are relatively unaffected by mutations contributing to antibody-evasiveness, and even less so following T-cell responses induced by past infections from heterologous variants [10,11,18]. The persistent high vaccine

effectiveness against severe Covid-19 has been attributed to the relative conservation of T-cell immunity from vaccination and natural infection, even when effectiveness against SARS-CoV-2 infection and mild Covid-19 have diminished because of antibody waning or relative antibody-evasiveness of some variants.

Our study only investigated quantitative T-cell responses following receipt of study treatment between HIV-uninfected participants and PWH and did not investigate qualitative differences in T-cell polyfunctionality and other cellular phenotypes. Another limitation of this study includes the small sample size of HIV-uninfected participants who were anti-N IgG seropositive at baseline, probably because of the different enrolment periods of the two HIV groups, which precluded comparisons between seropositive participants living with and without HIV. Also, the restrictive enrolment criteria of PWH, prevents generalizability to the overall black African population living with HIV in South Africa.

In conclusion, AZD1222 vaccination induced similar cellular immune responses between HIV-uninfected people and PWH on stable antiretroviral therapy. Additionally, hybrid immunity acquired through SARS-CoV-2 infection and vaccination, confer a heightened T-cell response that may result in protection against severe Covid-19; which is particularly important in countries where vaccines are belatedly rolled out only after a significant percentage of the population have developed immunity.

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Author contributions: W.C.M. and G.K. prepared this manuscript. S.A.M., A.L.K., J.G., L.F., C.L.C., G.K. and F.P. enrolled trial participants, as well as collected data and samples. W.C.M., C.K.M., N.J.M. and R.L. processed samples. W.C.M. and G.K. generated data. W.C.M., G.K. and A.I. analysed data by performing statistical analyses. W.C.M., G.K., C.K.M., A.L.K., J.G., L.F., F.P., W.A.B., M.C.N., C.L.C, S.C.G., T.L., A.J.P. and S.A.M. interpreted the results. All authors had full access to this study's data and had final responsibility for the decision to submit to publication.

Data availability: anonymized participant data will be made available upon request directed to the corresponding author.

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Conflicts of interest

There are no conflicts of interest.

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